Tissue Culture Notes

2017

Plant TC medium is used to grow and regulate plant cells. It contains macro-nutrients, micro-nutrients. Additional vitamin and PGRs are required if necessary

Macro-nutrients is a mixture of N, P, K soluble salts.

Micro-nutrients are salts of elements:

B, Cu, Fe, Mn, Mo, Zn.

Fertilizer Example:

20% Nitrogen Salts
10% Phosphate (P_2O_5)
20% Potash (K_2O)
0.016% B
0.06% Fe

Additional salts of Ca, Mg, S are required to allow cells to grow fast.

Some vitamins such as thiamin, inositol, and niacin help plant cells grow faster. Adding them to the medium will provide more nutrients.

Select a soluble fertilizer that has complete elements: N, P, K, Ca, Mg, B, Cu, Fe, Mn, Mo, Zn,

Avoid fertilizer that contains too much urea and phosphate because some plant cells don't want too much of them.

When plant cells grow in medium, they uptake salts and release ion hydrogen and ion hydroxide so medium becomes too acid or too basic

125 ppm Nitrogen is good enough to grow plant cells.

Normally ¼ - 1 teaspoon per gallon

OR

1 - 3 grams per liter.

A simple formula for carnivorous plants using fertilizer to make 1 liter.

30 grams Sugar

0.5 grams fertilizer 20-10-20
with micro-nutrient elements:
B, Mn, Mo, Fe, Cu, and NO UREA

10 ml vitamin solution,
dissolve 1 tablet multivitamin
in 100 ml water.

8 grams Agar

Add distilled water to make 1 liter solution

Adjust pH to 5.6

Components of TC growth medium

(ORGANICS : C, H, O)

Agar - neutral substrate

Sucrose - ($C_{12} \cdot H_{22} - O_{11}$) provides energy source for cells

Inositol - ($C_6 : H_{12} - O_6$) simple alcohol sugar

Hormones - control growth

fungicides - control contaminants

Antibiotics - control Contaminants

Antioxidants

(Inorganic Macro nutrients)

N - leaf growth, chlorophyll, amino acids, proteins, cell membranes

P - meristem growth, fatty cell membanes, DNA production

K - cell division, Root formation

S - Root development

Ca - Pectin (cell wall glue) vital to Meristem growth

Mg - Integral to chlorophyll molecules

Fe - Vital to pigment and chlorophyll formation

(Inorganic Micro nutrients)

B - Sugar movement within plants

Mo - Nitrogen fixation

Mn - Subtle (controls vital enzyme pathways)

Cu - Subtle (controls vital enzyme pathways)

Zn - Subtle (controls vital enzyme pathways)

Cl, Al, Na, Si, Co - (secondary; need for these Varies by species)

IBA - indolebutyric acid

NAA - naphthylacetic acid

IAA - indoleacetic acid

BAP - 6- Benzylaminopurine

MS - Murashige and Skoog medium

Components of a simple homemade media

1/8 cup table sugar = 30 grams

1 cup water

1/2 cup stock solution (Miracid diluted 1/4 tsp/gal) ← use only 1/4 cup if using 20-20-20 10-10-10

1/2 inositol tablet (125 mg)

1/4 Vitamin tablet with thiamin

2 TBS agar flakes

Note: This recipe is for general purpose use most carnivorous plants require a more dilute solution of nutrients.

Try reducing stock solution and sugar until best results are obtained.

500 ml. Agar Recipes

LMEA: 10 g. light malt extract
10 g Agar

MYA: 2 g. Brewers yeast
10 g. malt extract
10 g. Agar

.4/100 ml
2/100 ml
1.5-2/100 ml

DFA: Boil ½ cup dry dog food in 500 ml water. Strain the solution and add 10 g. Agar. Add water to 500 ml.

OVA: 10 g. ovaltine
10 g. Agar

FFA: 10 g. Fish Food Flakes
10 g. agar

RFA: 10 g. Rabbit food pellets
10 g. Agar

PDA - Potato Dextrose Agar

Take 300 g. potatoes. Cut them and boil them in ½ gallon water for 30 minutes on a simmer. The liquid will reduce to ~1 liter in that amount of time

Strain the liquid

Use 500 ml of the liquid.

Add 10 g Dextrose (Glucose)

10 g. Agar

You can Substitute Honey or Corn syrup for the Dextrose.

Fast Fred's PDA

200 g. potato infusion (dilute to 1 L. total)
OR use 4g. potato flakes

20 g. Dextrose

20 g. Agar

Add enough water to bring volume to 1 liter

Final pH 5.6 ± 0.2

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10 g. Agar, 5 g. Potato flakes,

10 ml honey or corn syrup

500 ml. water

Prepare Explants

Explants are the plant tissues to be cultured. Plant tissues that consist of meristematic cells (undifferentiated cells), have less cell walls or fibers, or are rich in cytoplasm (the material or protoplasm within a living cell, excluding the nucleus) are usually good candidates for explants.

Examples are root tips, shoot tips, young floral organs, immature embryos. young leaves and young stems.

large explants such as leaf blades and stems should be cut into smaller pieces to increase culture efficiency

Explants need to be surface Sterilized, This is usually done by soaking the explants in 10% bleach solution for 5-10 minutes.

Longer soaking time or higher bleach concentration usually causes cell damage to the explants and thus reduces the chance for a successful culture. A dip in a 70% ethanol may be done before soaking in bleach to reduce the surface tension so that bleach can easily make contact with the explants surface.

After sterilized in a bleach solution, the explants are rinsed three times in autoclaved water to remove bleach residue

Nitrogen is mostly provided in two forms - as nitrates and as ammonium compounds.

When nitrate is used alone, the pH of the medium drifts towards alkalinity, but adding ammonium compounds together with nitrate, checks the drift of pH.

Macro salts - X10 or X20

Micro salts - X 1,000

Murashige and Skoog medium

Major Salts : Macronutrients

Ammonium nitrate: NH_4NO_3 ← Substitute Sodium Nitrate or more Potassium Nitrate

1,650 mg/l.

Calcium Chloride: $CaCl_2 \cdot 2H_2O$
(damprid)
(ice melter)

440 mg/l

Magnesium Sulphate: $MgSO_4 \cdot 7H_2O$
(Epsom salt)

370 mg/l.

Monopotassium phosphate: KH_2PO_4

170 mg/l.

Potassium nitrate: KNO_3

1,900 mg/l.

Constituents	Amount (mg/L) of original medium	Amount (G.) X20 Stock
NH_4NO_3	1650	33.0
KNO_3	1900	38.0
$CaCl_2 \cdot 2H_2O$	440	8.8
KH_2PO_4	170	3.4
$MgSO_4 \cdot 7H_2O$	370	7.4

final Volume of Stock solution

1000 ml.

Make Calcium Chloride

$$CaCO_3\,(s) + HCl\,(l) \rightarrow CaCl_2\,(l) + CO_2\,(g) + H_2O\,(l)$$

Calcium carbonate → limestone, oyster shells, egg shells, chalk, marble.

KH_2PO_4 - Monopotassium phosphate is produced by the action of phosphoric acid on potassium carbonate.

Fert. grade = 52% P_2O_5 and 35% K_2O

NPK 0-52-34

Homemade media General Purpose

use ½ tsp for C.P. maybe ⅓ tsp.

1 teaspoon Peter's fertilizer (20-20-20)

2 tablespoon sugar

1 multivitamin pill (Do not crush- allow to partially dissolve only and then remove after a few minutes)

1 ml. PPM

Agar / water / hormones

Makes 1 liter.

Adjust pH to 5.5 - 6.0

N - P - K

Indicates the percentage by volume of Nitrogen (N), Phosphorus (P), and Potassium (K)

Nitrogen helps plants make the proteins they need to produce new tissues.

Phosphorus stimulates root growth, helps the plant set buds and flowers, improves vitality and increases seed size.
To absorb phosphorus, most plants require a soil pH of 6.5 to 6.8.

Potassium improves overall vigor of the plant. It helps the plants make carbohydrates and provides disease resistance. It also helps regulate metabolic activities.

There are three additional nutrients that plants need, but in much smaller amounts.

Calcium is used by plants in cell membranes, at their growing points and to neutralize toxic materials. In addition, calcium improves soil structure and helps bind organic and inorganic particles together.

Magnesium is the only metallic component of chlorophyll. Without it, plants can't process sunlight.

Sulfur is a component of many proteins.

Basic recipe for 200ml media

1.8 - 2.0 g. Agar

5.4g. Sugar

1.5 ml. Liquid fertilizer

18ml All natural Coconut water

180 ml. Water

Improved DIY media ~200ml

Added citric acid to the media to prevent and treat phenol exudates from oxidizing.

1.8 - 2.0 g. Agar

4.0 g. Sugar

1.0 ml. Liquid fertilizer pH 5.0 - 5.8

18ml. coconut water (Less than 10% concentration, greater concentrations caused callus death - pH 5.2)

180 ml water

1.0 mg/l IBA

50 mg/l Citrit Acid or less

Medium Stock Solutions

Before plant tissue culture formulations were commercially available, mineral salt mixtures were prepared as stock solutions ranging from 10 to 100 times the final concentrations used in the ~~each~~ medium.

Two solutions – one macronutrients
one micronutrients

These solutions must be kept fairly dilute (10-20X) to avoid precipitation of calcium and magnesium phosphates and sulfates.

Weight of molasses

$\frac{1}{4}$ tsp = 1.67 g.

1 tsp = 6.67 g.

1 TBS = 20 g.

Dark Corn Syrup

¼ tsp. = 1.71 g.

1 tsp = 6.83 g.

1 TBS = 20.5 g.

Weight of Honey

¼ tsp = 1.77 g.

1 tsp = 7.06 g.

1 TBS = 21.19 g.

Light corn Syrup

¼ tsp. = 1.67 g.

1 tsp = 6.75 g.

1 TBS = 20.24 g.

Using percentage by weight (w/v)

The formula for weight percent (w/v) is:

[mass of solute (g) / Volume of solution (ml) x 100]

1/4 tsp of molasses in 100 ml is only 1.67%

1/2 tsp : 3.33%

3/4 tsp: 5%

Percent %	PPM
0.01%	100 ppm
0.1%	1000 ppm
0.5%	5000 ppm
1%	10,000 ppm
2%	20,000 ppm
100%	1,000,000 ppm

.5% = 5000 ppm

To calculate grams/Liter of chlorine Powder

Grams/Liter = [% dilute / % concentrate] x 1000

Trichlor = 89% Available chlorine

[.5% / 89%] x 1000

5.618 g./L to make .5% OR 5000 ppm

* Trichlor isn't dissolving fast enough

Around 4 drops of 8.5% Bleach added to 33 ml. water Should give around 500 ppm chlorine.

Just under 2 ml in 33 ml Should give around 5000 ppm solution.

* Bleach had available Chlorine of 7.86% so calculations were not right.

Dichlor

Available chlorine 56%

Grams/Liter = [% diluter / % conc.] x 1000

= [.5 / 56] x 1000

.0089286 x 1000

8.929 g/Liter

.893 g/100 ml.

5000 PPM

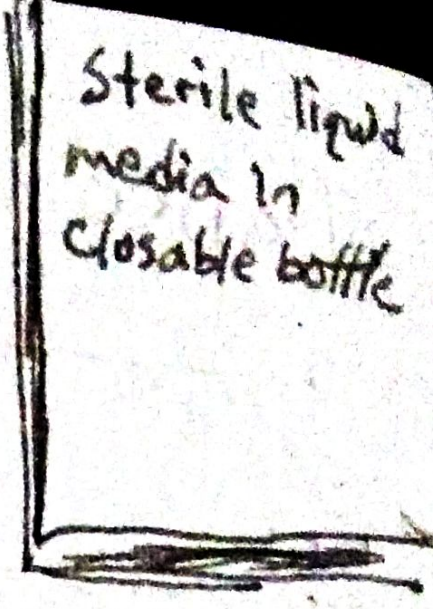
60 mg / 33 ml
to get 1000 ppm

~90 mg
to get
500 PPM
in 100 ml

~30 mg/33 ml
to get 500 ppm

About 5 crystals

Bill Graham's Pocket Tissue Culture Kit:



Contents: Screw-top tube, with Chlorine wash and Sterile liquid tissue culture multiplication media.

Add water to tube to 2/3 full to dissolve chlorine.

Trim cuttings to 1-1/2" and add to tubes.

Drain after one hour and add 2ml sterile liquid media

Kit supposed to make NaDCC (chlorine) solution around 500 ppm.

I'm now using 1000 ppm NaDCC for this method which is ~60 mg / 33 mL

Wash explant first Add NaDCC to tube and add 33 ml water.
Soak for 1 hour to clean both explant and tube or jar.

Drain liquid and add liquid media Med.
Soak bag of media in bleach or NaDCC
Cut open with sterile scissors or scalpel

NaDCC 56% Available Chlorine

25 ppm = 44 mg/L

500 ppm = 893 mg/L

1000 ppm = 1.785 g/L

2000 ppm = 3.571 g/L

4000 ppm = 7.142 g/L

5000 ppm = 8.928 g/L

NaDCC Calculations

$$\text{mg NaDCC} = \frac{\text{Liters water} \times \text{desired concentration (mg/L)}}{\frac{\text{Percent Chlorine of NaDCC}}{100}}$$

- .0025% = 25 PPM
- .05% = 500 PPM
- .1% = 1000 PPM
- .4% = 4000 PPM
- .5% = 5000 PPM

Take the % of Chlorine desired divided by the % of Chlorine in NaDCC times 1000 = Grams / Liter

- 1% = 1000 PPM
- 1/56 = .17857 [redacted]

[redacted] x 1000 = 1.785 g/L

Hydrogen Peroxide Spray

(~ 44 ml.)
One and a half ounces H_2O_2 (3%)
~~added~~ Per liter of water with
a drop of detergent

Dichlor - 2-5 g/L 20 min.

0.5 g/L 1-3 hr. presoak

Bleach Concentration in 33 ml.

@ 7.86% Available Bleach

500 ppm – 4 drops

1000 ppm – 8 drops

2000 ppm – 17 drops

2500 ppm – 1.050 ml (21 drops)

4000 ppm – 1.679 ml (34 drops)

5000 ppm – 2.099 ml (42 drops)

10,000 ppm – 4.198 ml (84 drops)

Water Purification by Bleach

Available Bleach	Drops per Quart/Gallon of clear water	Drops per liter of clear water
1%	10 per quart / 40 per gallon	10 per liter
4-6%	2 per quart / 8 per gallon (1/8 teaspoon)	2 per liter
7-10%	1 per quart / 4 per gallon	1 per liter

Shake and wait 30 minutes

Tap water typically has a chlorine concentration of 1 part per million (PPM) ~~Municipalities~~
Municipalities vary from 0.2 PPM to 4 PPM which is safe

Will not deactivate Giardia Lambia and Cryptosporidium

MSR MIOX WATER PURIFIER

Brine concentration between 60-80 g/L

1 Liter dose approx 2.75 mg/L of disinfectant

Test strips measure 4.0 to 5.0 ppm of free chlorine + or − 0.5 ppm.

Anode + 94% Platinum 6% Rhodium

Cathode − Titanium

grams calcium hypochlorite = (desired mg/L chlorine x gallons to be treated x 3.785 L/gal) / (1000 mg/ml x (% chlorine in HTH / 100))

Given this equation, if you figure the standard household bleach contains a 5% or 50,000 mg/L chlorine solution, it would be the following:

(50,000 x 1 x 3.785) / (1000 x .78) = 242

grams of your calcium hypochlorite per gallon of water to make an equivalent amount as one gallon of household bleach.

Dakin's Solution

Mix fresh
48 hrs. once opened
1 month sealed

used to treat infected wounds

diluted sodium hypochlorite solution 0.5%
~~5000 ppm solution~~

Measure 32 oz. (4 cups) of tap water.
Pour into clean pan

Boil 15 minutes with lid on pan

Add 1/2 teaspoon baking soda (1 tsp = 5 grams so 2.5 grams)

Clorox 5.25%	Full strength	1/2 Strength	1/4 Strength	1/8 Strength
	3 ounce 95ml	3 TBS + 1/2 tsp 48ml	1 TBS + 2 tsp 24 ml	2 1/2 tsp 12-14 ml
Water	32 oz 946.353 ml	32 oz	32 oz	32 oz

To make 50 ml. Dakin's Solution

125 mg. Baking Soda

either 0.446 grams (446 mg) NaDCC

OR

3.181 ml (64 drops) 7.86% Available chlorine Bleach

MS Stock Solution

Full pack MS dissolved in 60 ml water

Use 2.7 ml for a 45 ml. jar
for full strength

1/3 strength 0.9 ml or 18 drops

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for 6ml Batch, use ~7 drops for full strength

3.6 drops for 1/2 strength
2.4 drops for 1/3 strength

MS Stock in 45ml water

2.025 ml in 45ml full strength

.675 ml (13.5 drops) 1/3 strength

If only making 6ml.

5.4 drops / 6ml full

1.8 drops / 6ml 1/3 strength

Mix full pack of MS in 45ml Water to make stock solution.

How to convert Percent to ppm

1% = 10,000 ppm

So:

$$X_{(ppm)} = 10{,}000 \times X_{(\%)}$$

How to convert ppm to Percent

1% = 1/100

1ppm = 1/1,000,000

1ppm = 0.0001%

So:

$$X_{(\%)} = X_{(ppm)} / 10{,}000$$

DIY Miracle Grow

1 Gallon of water (3785.41 ml)

1 TBS Epsom Salt ~~(13.5g)~~ 15.5g.

1 tsp. Baking Soda ~~(4.4 grams)~~ 5.7g

½ tsp. Household Ammonia (2.46 ml) (~49 drops)

(~2 TBS)

Use one ounce of this mix with 3 cups water once a month.

Dilute 10-15 ~~gallons~~ 1 pt. Mix to 10-15 pt water

DIY Miracle grow

500 ml Water

1.78 g. Epsom Salt

.58 g. Baking Soda

6 drops Ammonia

dilute 1:10 - 1:15 for use

Try 1.78 g. Potassium Nitrate / 500 ml.

1.5 TBS Epsom Salt

1.5 tsp Baking Soda

0.25 - 0.5 tsp Ammonia

1 gallon water

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100 ml water

410 mg Epsom Salt

150 mg Baking Soda

1 drop Ammonia

Stock Solution Calculations

Volume of ~~insta~~ initial stock solution times the amount wanted divided by 1000 is the amount of stock needed

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Initial Stock Volume	100 ml
Final Volume Wanted	100 ml
Amount of Stock required	10 ml

$\sim$ 3/4 TBS OR 2 tsp

14.7868 ml = 1 TBS

4.92892 ml = 1 tsp

1 TBS = 3 tsp

Molecular weight of Sugar

342.2965 g/mol

Molecular weight salt

58.44277 g/mol

Chlorine Gas Sterilization

Put seed in small centrifuge tubes. 30-50 Arabidopsis Per tube.

100ml bleach in 250 ml beaker

Add tubes of seeds and the beaker of bleach to disiccator jar and add 3 ml concentrated 12 Molar HCl to the bleach and immediately close the desiccator.

- Allow sterilization to precede for 3-4 hours.

All this needs done in FUME HOOD or outside away from anything that could rust or corrode. Besides being toxic, Chlorine is very corrosive

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Culture Jar Ranking Code 0-4

Ø = No Stem or Roots
1 = Root or Stem Only
2 = Stem with some leaves
3 = Stem, Roots, Leaves
4 = Plantlet

Culturing from Seed V.F.T.

Wash seeds for 2 min. in running water
then use roughly 4000 ppm NaDCC
(1g/250ml) ← 2250ppm by my calc.
and pour a little into
the millipore filter. Wait 5 min. and
drain then put seeds in and pour
solution in ... if some float, you'll need
to stir them every minute to keep all ~~seeds~~
surfaces exposed.

← Change to 45 min.

Drain after 15 min. and put on 5.5 g agar/L.
1/2 MS or EX 3 only do a small amount and
wait a week. If good, do the rest otherwise increase
time to 30 min.
Only put 5 per jar and ~~sat~~ scatter them so
if one is bad, you can put the rest in another jar.
Score the surface of agar and put seeds on rough surface

Making Chlorine Gas

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manganese dioxide with 4 mole equivalents of hydrochloric acid.

Pool Chlorinator with appropriate amount of HCl.

Calcium hypochlorite + 4 mole equivalents HCl.

Dichlor/Trichlor + 3 mole equivalents HCl

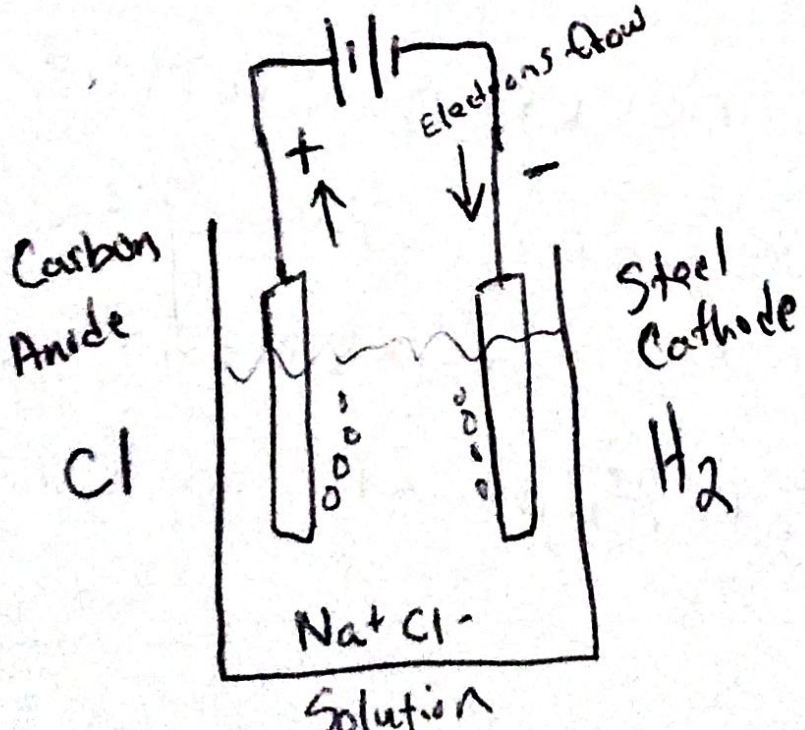
HCl can be substituted with equimolar mixture of sodium chloride salt and sodium bisulfate (pH lowering chem.)

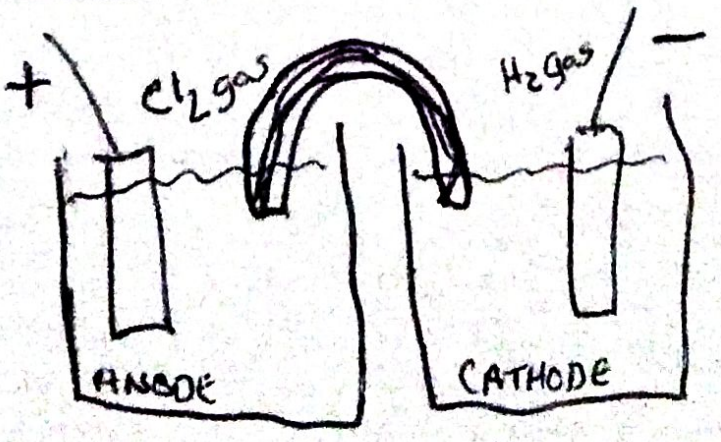
Test for Chlorine gas, 0.1 g Starch and 0.5 g potassium iodide in 20 ml water. Filter strips dipped in the solution will turn blue or brown in the presence of chlorine

Electrolytic Production of Bleach

$$NaCl_{(aq.)} + H_2O = Cl_{2(g)} + H_{2(g)} + NaOH_{(aq.)}$$

$$Cl_{2(aq.)} + NaOH_{(aq.)} = NaClO_{(aq.)} + NaCl_{(aq.)} + H_2O$$





Since a liter has a mass of 1000 grams, adding 10 grams of something to a liter is the adding of 1%

Urea should never be sterilized in an autoclave, along with other constituents, as it is unstable and breaks down at 15 psi steam pressure

Cordyceps Broth Media

1 tsp Azomite Powder = 4.72 grams
use 3.32 g/L

FOR 100 ml Broth

1.8 g Sugar (Sucrose)

0.5 g Corn Starch

0.6 g Yeast OR 1.4 g Crushed Chia Seeds

0.33 g Azomite Powder

Use 1 part Dry Grain and 2 Parts of the Liquid Broth per container

Pressure cook

1 TBS Molasses = 21.06 g

Sugars total = 15.74 g

Dollar Store Brand says only 13 g

Sucrose = 6.19 g

Glucose (dextrose) = 2.51 g

Fructose = 2.69 g

Calcium = 43 mg — Dollar Store 4% Daily Value

Iron = 0.99 mg — Dollar Store 6% Daily Value

Magnesium = 51 mg

Phosphoras = 7 mg

Potassium = 308 mg → Dollar Store says 170 mg

Sodium = 8 mg — Dollar Store 5mg

Zinc = 0.06 mg

Copper = 0.103 mg

Manganese = 0.322 mg

Selenium = 3.7 µg

Liquid Culture Recipe

Extra light Malt Extract — Yeast can be used as substitute

Karo light corn syrup

100 ml H_2O
2 ml Karo
0.2 g ME

150 ml water
3 ml Light Karo
0.3 g. ME

1 ml Karo weighs 1.5 g

1/4 tsp malt to 1 tsp Karo to 600 ml water

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3 tsp Karo for every 5 ml

1/4 tsp for 0.5 g malt

250 ml water

Venus Flytrap germination

Sphagnum moss rinsed of minerals
Run distilled water through it and let it drain out 4 times.

silica sand or perlite

Growing container - moist and warm with good air circulation.

Moisten growing medium with distilled water

Don't bury seeds. - very lightly covered

4-6 weeks

Bright Indirect light

Grown VFT require a lot of light 3-4 hrs good light minimum

Germination period 13-35 days
above 78°F up to 90°F

Converting Temperature to Kelvin

F to K

$$K = (y^{\circ}F + 459.67) \times \frac{5}{9}$$

$$K = \frac{(y^{\circ}F - 32)}{1.8} + 273.15$$

this gives °F to °C

Celsius from Fahrenheit

$$T_{(°C)} = (T_{(°F)} - 32) / 1.8$$

Spreadsheet Formula

= Convert (A1, "C", "F")

= Convert (A1, "F", "C")

= Convert (A1, "F", "K")

Temperature Converting

$$T_{(°F)} = T_{(°C)} \times 9/5 + 32$$

$$T_{(°F)} = T_{(°C)} \times 1.8 + 32$$

$$T_{(°C)} = (T_{(°F)} - 32) \times 5/9$$

$$T_{(°C)} = \frac{T_{(°F)} - 32 \times 5}{9}$$

$$T_{(°C)} = \frac{(T_{(°F)} - 32) \times 5}{9}$$

Sea Water is made up of primarily Sodium Chloride (approx. 84%) and Magnesium Sulfate (approx. 14%)

It's this magnesium which makes sea water hard.

The remaining 2% or so include all the trace elements.

Drosophila Melanogaster

Flightless fruit flies

Day Ø - flies added to fresh culture.

Day 8 - Media develops 2 distinct layers due to larvae.

Day 10 - Larvae migrate up the culture and pupate.

Day 12 - Pupae darken as flies develop.

Day 14 - Adult flies emerge

Day 28 - Dispose of culture